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Geographic variation in cold hardiness among eastern white pine (*Pinus strobus* L.) provenances in Ontario

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Abstract

Needle and shoot samples of eastern white pine provenances in Ontario were subjected to a series of artificial freezing tests to determine provenance differentiation in fall and winter cold hardiness. Results indicated that under the natural processes of cold acclimation in the fall, above ground tissues of eastern white pine could withstand low freezing temperatures in September–October that exceeded the local long-term minimum climate extremes of the same periods. The severity of cold damage increased at a faster rate in September than in October and November when temperature was further decreased below the thresholds that started to cause cold damage. Under the selected freezing temperatures that inflicted between 15 and 85% average needle or/and cambium damage, consistent results were observed among freezing tests that were conducted using samples of different ages and cold acclimation stages. A clinal pattern of geographic variation in the severity of cold damage was detected with a trend parallel to that of growth potential. As thermal conditions improve from the north to south in Ontario, the growth potential of a provenance increases but the degree of cold hardiness decreases. Based on risk assessments using a spatial regression approach, it was, however, believed that northward seed transfer within a distance of 2–2.5° in latitude could be acceptable to promote stand growth while incurring no statistically significant increase in cold damage.

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Keywords: *Pinus strobus*; Cold hardiness; Geographic variation; Seed transfer

1. Introduction

Cold hardiness is one of the most concerns for seed transfer between geographic regions in reforestation in the temperate and boreal forests (Rehfeldt, 1985, 1986; Thomas and Lester, 1992; Benowicz et al., 2001). Low survival and/or cold injury of southern provenances at more northerly sites can cause loss of forest productivity (Matyas and Yeatman, 1992). Information about genetic variation among provenances in cold hardiness is essential to formulate seed transfer guidelines.

Eastern white pine (*Pinus strobus* L.) has a large natural distribution in the eastern United States and Canada, ranging from Georgia to northern Ontario. Range-wide and regional provenance tests have shown the species to be widely genetically variable in growth, with provenances of 2–7° (latitude) south

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of a planting site frequently showing better performances (Fowler and Heimburger, 1969; Garrett et al., 1973; Genys, 1987; Li et al., 1997; Joyce et al., in press). Interests, thus, arise to increase forest growth through northward seed transfer in reforestation programs.

Risks of cold damage in relation to northward seed transfer in eastern white pine have, however, not been well evaluated. Uncertainty remains about safe seed transfer distances since little is known about patterns of geographic genetic variation in cold hardiness and the lack of provenance trials established at more northerly sites. In Ontario, a short-term regional provenance test with extensive samples of eastern white pine provenances has revealed considerable geographic variation in growth, but not in cold damage (Joyce et al., in press). Because climatic conditions at the planting sites during the testing period were relatively mild, no conclusion has been drawn from the field observations on provenance differences in cold hardiness.

An artificial freezing test was shown effective in revealing genetic variation in cold hardiness on several species (Rehfeldt, 1986; Thomas and Lester, 1992; Aitken and Adams, 1996, 1997; Anekonda et al., 2000). An advantage of an artificial freezing test is that it can simulate different levels of cold severity and detect the threshold temperatures that start to cause cold damage at a given cold acclimation stage. Comparing the threshold temperatures of different provenances with long-term climate extremes of a reforestation region at similar tree growing stages could provide references to predict the potential risks from northward seed movement (Timmis et al., 1994).

In this paper, we report the results of artificial freezing tests on shoot and needle samples taken from more than 110 eastern white pine provenances in Ontario at different growing stages and in different years. Our objectives were to: (1) detect the threshold temperatures that start to cause cold damage; (2) detect the scale and patterns of geographic variation among provenances in the severity of cold damage under selected freezing temperatures; (3) assess the potential risks of northward seed transfer in Ontario by comparing the observed threshold temperatures with climate extremes of the reforestation regions.

2. Material and methods

2.1. Materials and freezing conditions

Tissues of 112 eastern white pine provenances in Ontario (Fig. 1) were artificially frozen using needles taken from 2-years-old greenhouse grown seedlings in 1996, and detached shoots and needles from 6- to 7-years-old trees in a provenance trial established in Sault Ste. Marie during 2000 and 2001. Methods of sampling for natural eastern white geographic populations, seedling culture and field provenance trial establishment were detailed elsewhere (Joyce et al., in press).

In 1996, needles of 2-years-old seedlings were collected on September 3, 5 and 7, respectively, from three replications of a greenhouse study that used a randomized complete block design with 10-seedling plots. At the time of sample collection, seedlings were cultivated under a temperature regime of 28 °C (day)/18 °C (night). Collected needle samples were conditioned at 2 °C in plastic bags before freezing that occurred between September 5 and 12. During the artificial freezing, temperature was lowered at 5 °C/h till reaching a target temperature, holding for 2 h, and then gradually increased to room temperature at 5 °C/h using a model SM-32-C Thermotron. The target temperatures were chosen at −7, −9 and −11 °C, respectively, based on pre-freezing test results. After freezing, needle samples were retained in plastic bags for approximately 10 days for damage symptom development and, then, visually scored as healthy (less than 5% needle damaged), lightly damaged (5–20% needle damaged) and heavily damaged (>20% needle damaged).

In the first series of freezing tests of detached shoots, healthy current year shoots with well-developed terminal buds of approximately 10 cm long, together with needles were collected from 6-years-old trees in a provenance trial in Sault Ste. Marie, between September 25 and 28, 2000. The trial was established in 1996 using 1-year-old seedlings and a randomized complete block design with 10-tree row plots. The same eastern white pine provenances that were used in the greenhouse seedling freezing tests were included in the field provenance trial. Shoots were collected from lateral branches of the first whorl from the top, approximately 1.5–2.5 m

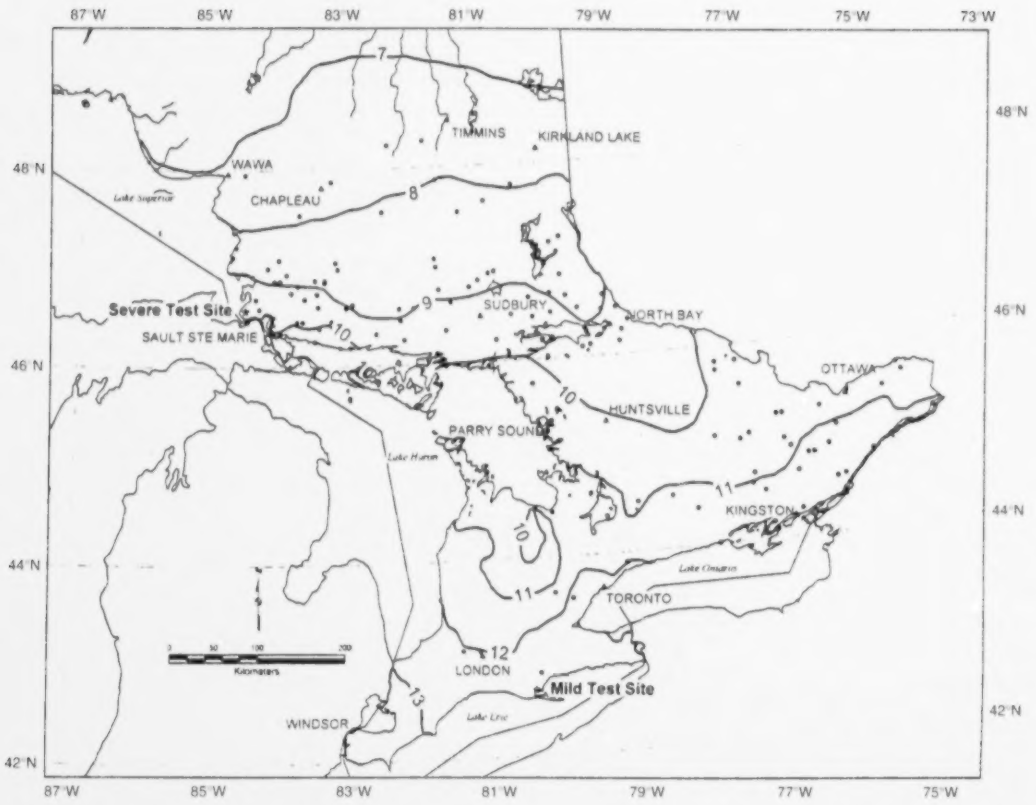

Fig. 1. Distribution of provenance samples (●) town city (▲), field provenance test site (★), and the annual MAXTs (°C) (i.e. the numbered lines) in Ontario.

above the ground and the same crown aspect within a block.

Under each freezing treatment (temperature) level, a provenance was represented by up to 10 shoots comprised of one shoot per tree from a 10-tree field row plot within a replication. Shoots of each provenance were packaged into a plastic bag and sprayed with moisture at harvest. Shoot samples were stored at 2 °C before freezing, which occurred within 3 days of sample collection. Freezing was performed using the same SM-32-C Thermotrons. Pre-freezing tests were conducted using randomly collected shoot samples to search for the temperatures that started to cause cold damage. Consequentially, target temperatures were set below the thresholds for the formal freezing tests at 3–5 °C intervals. During freezing, temperature was lowered at 3 °C/h until reaching a target temperature, holding for 5 h, and then increased to 2 °C at 3 °C/h. Frozen tissues were maintained at 2 °C for about 24 h for ice to thaw and, then, spread out on a laboratory bench at room temperature. The target freezing temperatures are shown in Table 1. To monitor the uniformity and stability of reaching the target temperatures, multiple temperature sensors were placed inside plastic bags containing shoot samples and at different positions in the freezing chamber. Damage was visually scored 8–10 days after freezing based on color changes of needles and dissected cambium surfaces of each shoot, following the established methods (Aitken and Adams, 1996; Anekonda

Table 1
Average percentage of needle and cambium damage under different artificial freezing temperatures and cold acclimation stages indicated by the date of sample collection

Freezing date	T^a (°C)	Needle (%)			Cambium (%)	
		Healthy	Light damage	Severe damage	Healthy	Damaged
September, 1996 (greenhouse seedlings)	−7	86.9	13.1	0.0	–	–
	−9	36.9	25.5	37.5	–	–
	−11	6.1	11.2	82.7	–	–
		Healthy	Damaged			
September, 2000 (detached shoots from 6-years-old trees)	−16	84.3	15.7	–	99.1	0.9
	−19	67.0	33.0	–	89.5	10.5
	−22	11.5	88.5	–	70.1	29.9
	−25	22.1	77.9	–	69.0	31.0
	−30	6.5	93.5	–	64.0	36.0
	−35	0.2	99.8	–	58.1	41.9
	−40	0.0	100.0	–	45.9	54.1
September, 2001 (detached shoots from 7-years-old trees)	−11	100.0	0.0	0.0	–	–
	−14	51.1	31.2	17.7	–	–
	−17	15.4	30.3	54.3	–	–
	−20	0.0	0.0	100.0	60.0	40.0
October, 2001 (detached shoots from 7-years-old trees)	−19	80.7	19.3	0.0	100.0	0.0
	−22	51.7	40.9	7.4	100.0	0.0
	−25	41.5	35.0	23.5	100.0	0.0
	−28	38.2	43.3	18.5	100.0	0.0
November, 2001 (detached shoots from 7-years-old trees)	−31	89.3	10.7	0.0	100.0	0.0
	−34	85.8	14.2	0.0	100.0	0.0
	−37	70.9	28.5	0.5	100.0	0.0
	−40	75.3	24.7	0.0	100.0	0.0

[a] Target freezing temperatures.

et al., 2000). Each shoot was scored by needle and/or cambium as healthy (no damage, colored green) or damaged (yellowed or browned).

In 2001, freezing tests were repeated using detached shoots from 7-years-old trees of the same provenance trial in the middle of September, October, and November, respectively. Freezing test procedures were the same as in 2000. Date of sample collection, field air temperatures recorded from an onsite weather station, and the freezing target temperatures are shown in Fig. 2. After freezing, needle damage was scored as healthy (no obvious damage, 95% colored green), lightly damaged (yellowed $< 40\%$) and severely damaged (browned or yellowed $> 40\%$) and cambium damage scored as healthy (no damage, colored green) or damaged (yellowed or browned).

2.2. Statistical analysis

Raw data for each provenance under each freezing treatment was presented as percentage of healthy or damaged tissues with the experimental unit being a plot of the field provenance test. The data transformation method of $y = \arcsin(\sqrt{x})$ was used to stabilize variances (Rawlings, 1988) before all statistical analyses, where, y is the transformed score and x is the original plot damage percentage. When needle scores of light and severe damage were analyzed jointly, an index was used that weighed the transformed scores of light and heavy damage by 1 and 2, respectively, on a per plot basis.

Analysis of variance (ANOVA) and variance component estimation were performed using SAS GLM and Mixed procedures (SAS Institute Inc., 1996),

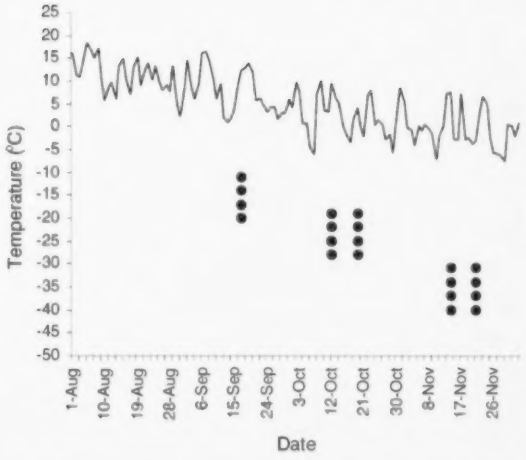

Fig. 2. Daily minimum temperature 1.5 m above ground at site of the field provenance test in Sault Ste. Marie in 2001 and the shoot sample collection date and freezing temperatures (filled circles) used in the study.

respectively, for each freezing test with a linear model:

$$y_{ijk} = \mu + R_i + T_j + RT_{ij} + P_k + RP_{ik} + TP_{jk} + e_{ijk}$$

where, y_{ijk} is the transformed score of the kth provenance frozen under the jth target temperature of the ith replication, μ the overall mean, R_i the ith replication, T_j the jth freezing temperature, RT_{ij} the interaction between the ith replication and the jth freezing temperature, P_k the kth provenance, RP_{ik} the interaction between the ith replication and the kth provenance, TP_{jk} the interaction between the kth provenance and the jth freezing temperature, e_{ijk} the residual of the kth provenance frozen at the jth target temperature of the ith replication.

In the analysis, temperature was treated as a fixed effect, and replication and provenance as random. Appropriate terms were dropped from the above model when analyses were performed under individual temperature or replication levels.

2.3. Patterns of genetic variation

Patterns of geographic variation were detected using multiple regression approaches. Independent variables were geographic coordinators (i.e., latitude, longitude and elevation) and climatic variables of provenance origin, which were interpolated from a climate model of Ontario (McKenney et al., 1999). Dependent variables were the marginal means of transformed damage scores of needle or cambium across freezing temperature levels at a given cold acclimation stage (e.g., mean needle damage of greenhouse seedlings (ND_GRH), mean needle damage of detached shoots in September 2000 (ND_00S), mean cambium damage of detached shoots under freezing temperature range from −19 to −25 °C in September 2000 (CD_00Sa), mean cambium damage of detached shoots under freezing temperature range from −30 to −40 °C in September 2000 (CD_00Sb), mean needle damage index of detached shoots in September 2001 (ND_01S), mean cambium damage of detached shoots in September 2001 (CD_01S), mean needle damage index of detached shoots in October 2001 (ND_01O) and mean needle damage index of detached shoots in November 2001 (ND_01N)). The backward regression analysis option (SAS Institute Inc., 1996) was used for independent variable selection for the full models. Simple regression was later performed for cold damage against the most prominent environmental variables to simplify model interpretation.

2.4. Risk assessment

Factors most likely to influence cold damage at a northerly site on trees of southern provenances include: (1) genetic variation among provenances in cold hardiness; (2) the steepness (i.e., regression slope) of cold hardiness changes against the independent geographic/climatic variables in a spatial variation model; (3) the seed transfer distance. Rehfeldt (1979, 1986) proposed the so-called LSD(0.2)/b approach to determine the safe distance for seed transfer under a simple regression model, which was followed in this study. In the LSD(0.2)/b method, the safe seed transfer distance is calculated in three steps: (1) provenance means are regressed against a prominent environmental variable (e.g., geographic coordinators or climatic variables) and the regression slope b is obtained; (2) the least significant difference among provenance means is calculated at the 80% significant probability level (i.e., LSD(0.2)) through an ANOVA and multiple comparison; (3) the safe seed transfer distance is calculated as the ratio of LSD(0.2) to b.

3. Results

3.1. Cold damage and scales of variation

Needles of 2-years-old seedlings from the greenhouse in early September showed cold damage at −7, −9 and −11 °C. Healthy needles reduced to 86.9% at −7 °C, 36.9% at −9 °C and 6.1% at −11 °C (Table 1). ANOVA indicated significant differences among provenances in the transformed scores of healthy, lightly and severely damaged needles. Provenance accounted for 25.5% of the total phenotypic variation in healthy needles at −7 °C, but only 7.6% at −11 °C. In contrast, provenance accounted for 0% of phenotypic variation in severely damaged needles at −7 °C and that increased to 21.8% at −11 °C.

For the detached shoots frozen in late September 2000, needle damage occurred at higher temperatures than cambium damage did with a threshold at about −14 °C. Needle damage increased from 15.7% to nearly 94% as temperature decreased from −16 to −30 °C. Cambium damage started to appear at −19 °C (10.5%) and substantially increased in the temperature range of −30 °C (36%) to −40 °C (54.1%). Differences among provenances in needle and cambium damage were significant under certain freezing temperatures, but not for all freezing temperature levels because, as a binomial trait expressed in percentage, both among and within provenance variation became less detectable when damage was either close to nil or 100%. Under desirable freezing temperatures where cold damage was closer to 50%, provenance variation accounted for up to 20.6% in needle damage and 26.9% in cambium damage of the total phenotypic variances (Table 2).

Repeated freezing tests in middle September 2001 showed similar results to those in late September 2000. No needle or cambium damage was observed at temperature as low as −11 °C, but damage increased to nearly 100% for needles and 40% for cambium at −20 °C. Under the freezing temperatures of −14 and −17 °C, up to 19.4 and 23.9% of phenotypic variations in healthy needle and severely damaged needle were, respectively, accounted by

Table 2
Variance components contributing to total phenotypic variation in cold damage under different artificial freezing temperature ranges and tissue cold acclimation stages[a]

Freezing time	Trait[b]	Freezing temperature range	σ_p^2 (%)	σ_{tp}^2 (%)	σ_e^2 (%)
September, 1996	NH	−7, −9, −11	22.6	0.0	77.4
	LDN	−7, −9, −11	22.9	0.0	77.1
	SDN	−7, −9, −11	0	7.0	93.0
	INDN	−7, −9, −11	24.6	0.0	75.4
September, 2000	DN	−16, −19, −22, −25	20.6	0.0	79.4
	DC	−19, −22, −25	14.8	0.0	85.2
	DC	−30, −35, −40	26.9	0.0	73.1
September, 2001	HN	−14, −17	19.4	0.0	80.6
	LDN	−14, −17	0	15.1	84.9
	SDN	−14, −17	23.9	0.0	76.1
	INDN	−14, −17	24.0	0.0	76.0
	DC	−20	8.0	–	92.0
October, 2001	HN	−19, −22, −25, −28	30.7	0.0	69.3
	LDN	−19, −22, −25, −28	11.2	0.0	88.8
	SDN	−19, −22, −25, −28	12.6	0.0	87.4
	INDN	−19, −22, −25, −28	31.0	0.0	69.0
November, 2001	HN	−31, −34, −37, −40	7.7	0	92.3
	LDN	−31, −34, −37, −40	7.7	0	92.3

[a] Statistical analysis is based on transformed plot data.

[b] NH: healthy needle, DN: damaged needle, LDN: lightly damaged needle, SDN: severely damaged needle, INDN: index of needle damage, DC: cambium damage. σ_p^2, σ_{tp}^2 and σ_e^2 are the estimates of variance components for provenance, provenance–temperature interaction and residual, respectively.

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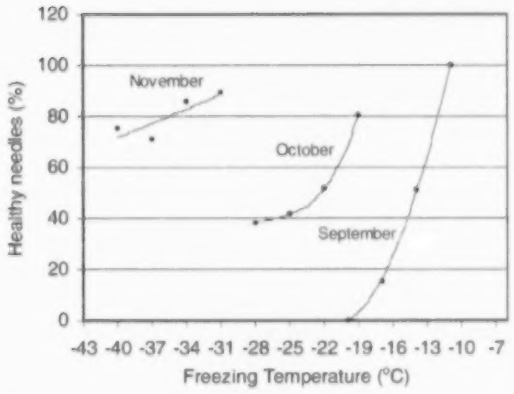

Fig. 3. Decreasing freezing temperature increased cold damage at a steeper rate in September than in October and November.

provenance differences. At −20 °C, provenance, however, accounted only for about 8% of phenotypic variation in cambium damage, which was lower than that in September 2000.

For the freezing tests in mid-October and November 2001, no cambium damage was scored under the applied freezing temperatures. Only light needle damage was scored at −19 °C in mid-October and −31 °C in mid-November (Table 1). Although needle damage increased as freezing temperature decreased, the rate was much slower as compared with that in September. For instance, when temperature was further lowered for approximately 9 °C below the thresholds that started to cause injury, needle damage increased from 0 to 100% in September, but only from 19.3 to 61.8% in October, and from 10.7 to 24.7% in November, respectively (Fig. 3). Furthermore, no severe needle damage was observed in mid-November, indicating most needles and almost all shoot cambium were able to tolerate as low a temperature as −40 °C in Sault Ste. Marie at that cold acclimation stage.

3.2. Correlation among cold damage traits

Observed differences in freezing damage among provenances were partially repeated among different freezing tests with varying freezing temperatures, tree ages and cold acclimation stages. For instance, provenance differences in needle damage observed on the 2-years-old greenhouse seedlings were significantly correlated with needle and cambium damage scored on samples from 6- to 7-years-old trees in the field provenance trial (Table 3). Stronger correlation was detected among cambium damage assessed at the same or different years under different freezing conditions. Provenance–temperature interaction was generally insignificant. Needle damage scored in November 2001 was, however, not correlated with needle or cambium damage assessed at other freezing test periods because of little provenance differentiation at that cold acclimation stage. With no exception,

Table 3
Pearson correlation matrix among provenance mean needle and cambium damage of different artificial freezing tests and the 6-years-old field provenance mean height

Trait[a]	ND_GRH	ND_00S	CD_00Sa	CD_00Sb	ND_01S	CD_01S	ND_01O	ND_01N
ND_00S	0.128							
CD_00Sa	0.343**	0.678**						
CD_00Sb	0.403**	0.204*	0.532**					
ND_01S	0.363**	0.214*	0.318**	0.430**				
CD_01S	0.227*	0.408**	0.555**	0.610**	0.437**			
ND_01O	0.311**	0.322**	0.271**	0.329**	0.264**	0.227*		
ND_01N	0.002	0.108	0.104	0.065	0.063	0.025	0.160	
6-YR_HT	0.302**	0.291**	0.410**	0.508**	0.225*	0.304**	0.322**	0.153

[a] Mean needle damage of greenhouse seedlings (ND_GRH), mean needle damage of detached shoots in September 2000 (ND_00S), mean cambium damage of detached shoots in September 2000 under freezing temperature range −19 to −25 °C (CD_00Sa) and under freezing temperature range −30 to −40 °C (CD_00Sb), mean needle damage index of detached shoots in September (ND_01S), October (ND_01O), and November (ND_01N), 2001, mean cambium damage of detached shoots in September (CD_01S), and the 6-years-old provenance mean height (6-YR_HT). Needle and cambium damage is based on transformed scores.

* Significant at the probability level of $\alpha = 0.05$.

** Significant at the probability level of $\alpha = 0.01$.

Table 4
Prediction models of cold damage of eastern white pine provenances and the northward seed transfer distances under different artificial freezing test conditions

Trait[a]	Full model		LSD(0.2)[b]	Simple regression model against								Transferable distance (LSD(0.2)/*b*)			
	R^2	Environmental variable[c]		MAXT		MT		MINT		GSL[d]		MAXT (°C)	MT (°C)	MINT (°C)	GSL (day)[d]
				R^2	*b*	R^2	*b*	R^2	*b*	R^2	*b*				
ND_GRH	0.350	ADD1, ADD2, ADD3, ADD4, JDS, JDE, MINT, MAXT, MT	0.133	0.175	0.059	0.174	0.045	0.175	0.036	0.159	0.0047	2.25	2.96	3.69	28.3
ND_00S	0.159	ADD3, TP1, TP3	0.165	0.113	0.043	0.107	0.032	0.103	0.025	0.100	0.0034	3.84	5.16	6.60	48.5
CD_00Sa	0.286	MAXT	0.149	0.286	0.053	0.134	0.040	0.274	0.032	0.272	0.0043	2.81	3.73	4.66	34.7
CD_00Sb	0.361	JDS, TP1, TP4, MTRP3	0.237	0.303	0.099	0.300	0.075	0.291	0.059	0.305	0.0083	2.39	3.16	4.02	28.6
ND_01S	0.275	ADD2, ADD3, ADD4, MINT, MTRP3, TP2, TP3, TP4	0.639	0.083	0.127	0.065	0.086	0.056	0.063	0.062	0.0092	5.03	7.43	10.1	69.5
CD_01S	0.240	ADD4, JDE, MAXT, MTP3	0.884	0.152	0.189	0.138	0.137	0.128	0.105	0.127	0.0145	4.68	6.45	8.42	61.0
ND_01O	0.174	ADD2, ADD3, ADD4	0.232	0.129	0.081	0.137	0.064	0.136	0.051	0.136	0.0070	2.86	3.63	4.55	33.1

[a] The definition of traits is the same as in Table 3.

[b] Least significant distance among provenance means at the probability level of $\alpha = 0.2$ in a multiple comparison analysis.

[c] Climatic variables are: aggregated degree days in the growing period (GP) 1 (ADD1), 2 (ADD2), 3 (ADD3), and 4 (ADD4), respectively; annual mean minimum (MINT), mean (MT) and mean maximum (MAXT) temperatures; Julian day of growing season start (JDS) and end (JDE); mean temperature in GP3 (MTP3); mean temperature range in GP3 (MTRP3); total precipitation in GP1 (TP1) and in GP3 (TP3). Where, GP1: before growing season start, GP2: the first 6 weeks of growing season, GP3: the whole growing season, and GP4 = GP3 − GP2.

[d] Growing season length.

provenance mean scores on cold damage were significantly positively correlated with the 6-years-old provenance mean height in the field provenance trial.

3.3. Patterns of genetic variation in cold damage

A clinal pattern of variation in cold damage was detected among eastern white pine provenances in Ontario. Linear relationships between cold damage and geographic and climatic variables of the provenance origins were evident. Due to the high degree of auto-correlation among most of the climatic and geographic variables in eastern Ontario where the eastern white pine provenances were sampled (Fig. 1) (data not shown), different sets of independent variables were selected to predict cold damage for different cold hardiness traits (Table 4). These selected prediction variables were, however, different presentations of the thermal conditions of the provenance origins. The similar nature among those selected prediction variables permitted a reduced model to simplify interpretation. In general, as the thermal conditions improve (i.e., higher temperature and longer growing period) from north to south in Ontario, provenances tended to suffer more cold damage under freezing conditions that were severe enough to cause appreciable damage (>15%) to the mixture of samples of all provenances (Fig. 4). Among the individual independent variables, the annual mean maximum temperature (MAXT) of provenance origins was most prominent, which had the highest correlation with several needle and cambium damage scores assessed at different freezing dates and tree ages. Following the MAXT, the annual mean temperature (MT), annual mean minimum temperature (MINT) and the growing season length (GSL) were the close competitors (Table 4).

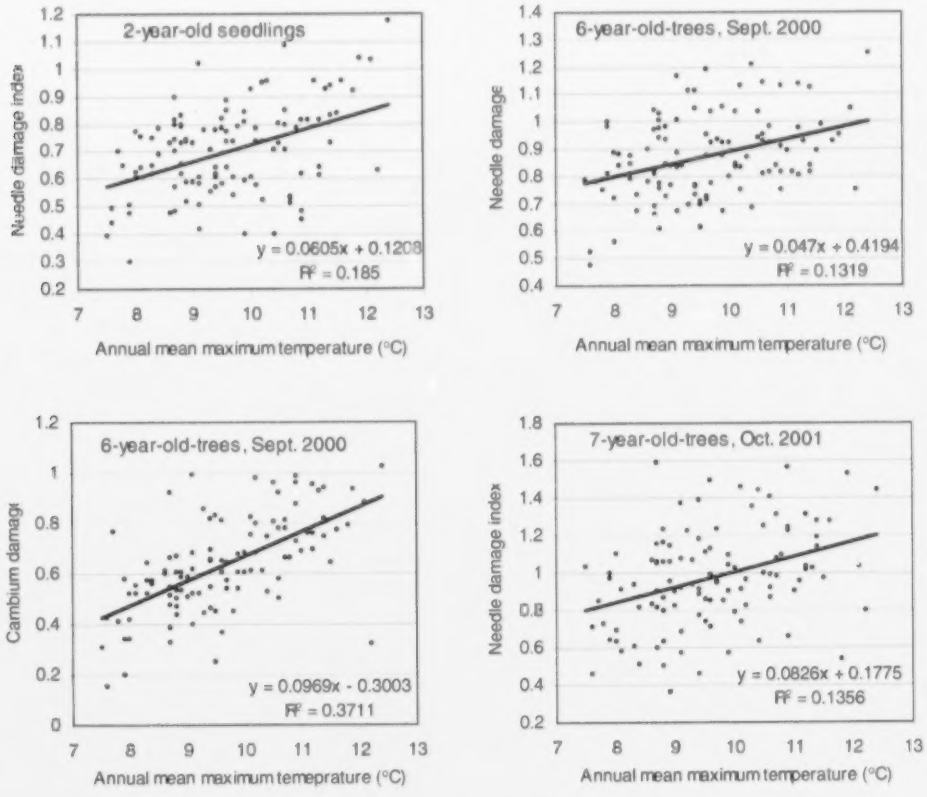

Fig. 4. Relationships between cold damage severity and the annual MAXT at provenance origins.

3.4. Risk assessment for northward seed transfer

Risks associated with northward seed transfer are given in Table 4, expressed as the units of the independent variables (i.e., LSD/*b*) beyond which seed transfer is likely to cause significant increase in cold damage at the 80% probability level. Based on the simple regression models (Table 4), it was predicted that when the differences in MAXT, MT, MINT and GSL between a provenance origin and a planting site do not exceed 2.0, 2.9, 3.6 °C and 28 days, respectively, there would be no significant increase in cold damage under the freezing conditions specified in this study. This is approximately converted to a 2–2.5° latitude distances over the landscape in eastern Ontario.

4. Discussion

Like other temperate and boreal forest species, eastern white pine provenances in Ontario showed considerable tolerance to low temperatures in the early fall and winter. Under the natural cold acclimation processes with daily minimum temperature ranging from 2 to 15 °C in August to middle September in Sault Ste. Marie, needle and shoot samples of white pine provenances were able to withstand temperatures as low as −11 °C in middle September. This level of cold tolerance has exceeded the local low temperature extreme since 1955 (−8.3 °C) in September and may have explained why several southern provenances more than 2.0° (latitude) south of the planting site could grow well in Sault Ste. Marie for the past 7 years. Such cold tolerance capacity is similar to that reported on other northern forest species. For example, in western white pine (*Pinus monticola*), freezing temperatures of −11 and −20 °C caused 4 and 82% of needle damage, respectively, in September (Rehfeldt et al., 1984). For lodgepole pine (*Pinus contorta*) in the northern Rocky Mountains, temperatures causing 50% needle injury were approximately −15 °C while the daily minimum air temperature was above 5 °C in middle September (Rehfeldt, 1980). Still, a study in Douglas-fir (*Pseudotsuga menziesii*) in west Oregon suggested that temperatures inflicting 20–80% of needle damage was at least 7–10 °C lower than the daily minimum air temperature of the same period (Aitken and Adams, 1996). Cambium damage in this study was inflicted at least 3 °C lower than the temperature under which needle damage occurred, suggesting stronger survival ability from cold damage for shoots than needles. Cold tolerance capacity is, however, heavily influenced by the stage of cold acclimation. For instance, in the greenhouse where seedlings were cultivated under temperature regimes of 28 °C (day)/18 °C (night), a relatively higher temperature such as −9 °C in early September had caused considerable needle damage in this study (Table 1).

The sensitivity of eastern white pine to cold temperatures decreased from September to November. Cold damage corresponding to temperature changes was more dramatic in September than in November. For instance, in September, a further decrease of freezing temperature by 3 °C below the threshold temperature had increased average needle damage by 30–50% as compared with 10–29% in October and around 5% in November (Fig. 3). Consequently, provenance variation in cold damage was larger in September and October than in November (Table 2). This has suggested that the most critical period to evaluate the risks associated with seed transfer is between September and October. Given sufficient time and low minimum daily temperature for cold acclimation, the difference among eastern white pine provenances in fall cold hardiness tended to diminish. Similar results had been reported on other species (Rehfeldt, 1980; Aitken and Adams, 1996).

Under selected freezing temperatures, substantial variation was detected among eastern white pine provenances in needle and cambium damage (Table 2). Consistent results were obtained from freezing samples of different ages, different cold acclimation stages, and different freezing temperatures as indicated by the significant Pearson correlation coefficients among provenance means (Table 3) and by the negligible provenance–temperature interaction. This may indicate a similar developmental stage of 2–7-years-old eastern white pines and the inherited differences among provenances as a result of adaptation to local environmental conditions.

The clinal pattern of geographic variation in cold damage among eastern white pine provenances is parallel to that for growth potential (Joyce et al., in press). Tree growth potential generally increases for seed sources from north to south in Ontario, so does

the severity of cold damage. The negative correlation between growth potential and cold hardiness necessitates a compromise in seed sources planning so that as much as possible gain in growth potential can be captured through northward seed transfer while keeping the risks of cold damage at limited levels. Although the north to south trend of increasing cold damage at a northern site was not as strong as that for growth potential as indicated by the R^2's in the fitted spatial models (0.63 in growth versus 0.36 in cold damage), it may, nevertheless, reveal inherited differences among the provenances. The weaker spatial pattern in cold damage may have possibly been affected by some less than ideal level of damage percentage (an ideal level of damage is 50%). For example, not all of freezing temperatures in this study had inflicted damage close to 50%. Thus, as a binomial trait, the revealed scale of variation among provenance in cold damage could be smaller than that could be when the incident levels were close to 50%. Also, visual scoring of cold damage was more subjective than measuring tree height against a ruler, which may result in lower experimental precision for cold damage assessment than for height.

The pattern of geographic variation in cold damage among eastern white pine provenances, nevertheless, provided useful information for assessing the risks associated with seed transfer under the circumstances. While cambium damage is vital to stem and tree survival, needle damage can cause reduced photosynthesis rates and forest productivity. Thus, in assessing the risks associated with seed transfer, it might be more appropriate to highlight the most serious scenarios of cold damage to determine the safe seed transfer distances regardless of whether the damage involves needle or cambium. According to the prediction equations fitted in this study, it seemed that a difference in annual MAXT between the provenance origins and a planting site of more than 2.5 °C would likely cause a statistically detectable increase in cold damage at the 80% of the probability level. Thus seed transfer is not recommended beyond that distance. Due to the strong linear correlation between MTs and latitude and GSL in eastern Ontario ($r = 0.96$, 0.98, respectively) the safe northward seed transfer distance for eastern white pine can be converted approximately to 2.5° in latitude or 28 days in GSL. Within this distance, seed transfer would be safe based on the freezing results. Additional evidences supporting this assessment are the threshold temperatures that started to cause needle damage in mid-September 2000 and 2001. The threshold temperatures were lower than the long-term extreme minimum temperatures in September in Sault Ste. Marie since 1955, which was −8.3 °C (from Canadian climate normals). That suggests that there is room for the southern provenances to withstand even lower ambient air temperatures.

While the patterns of geographic variation revealed under selected low temperatures are valuable to understand the inherited differences among provenances, defining the threshold temperatures that start to cause cold damage at different cold acclimation stages may be of more operational significance to help assess the risks associated with northward seed transfer. In this study, provenance differences were detected at temperature levels mostly well below the long-term local low temperature extremes of the same periods. The spatial patterns of provenance variation revealed under such low temperatures may not necessarily be expressed in the field where ambient air temperatures are higher. Thus, the recommended seed transfer distance based on the artificial freezing tests in this study could be deemed as a cautious approach to keep low risk of cold damage from seed movement. Alternatively, threshold temperatures that start to cause cold damage can be determined for different provenances under different cold acclimation stages to evaluate the risks of seed transfer with an injury prediction model (Timmis et al., 1994). Long-term climatic extremes are available for most parts of Canada and climate models can be developed to interpolate extreme low temperatures at different tree growing stages for a specific region. Searching through climate maps based on low extreme temperatures against the threshold temperatures can identify the safe areas for seed transfer of different provenances.

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